SI: Pentablock Thermoresponsive Hydrogels for Chemotherapeutic Delivery in a Pancreatic Cancer Model

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List of Figures:

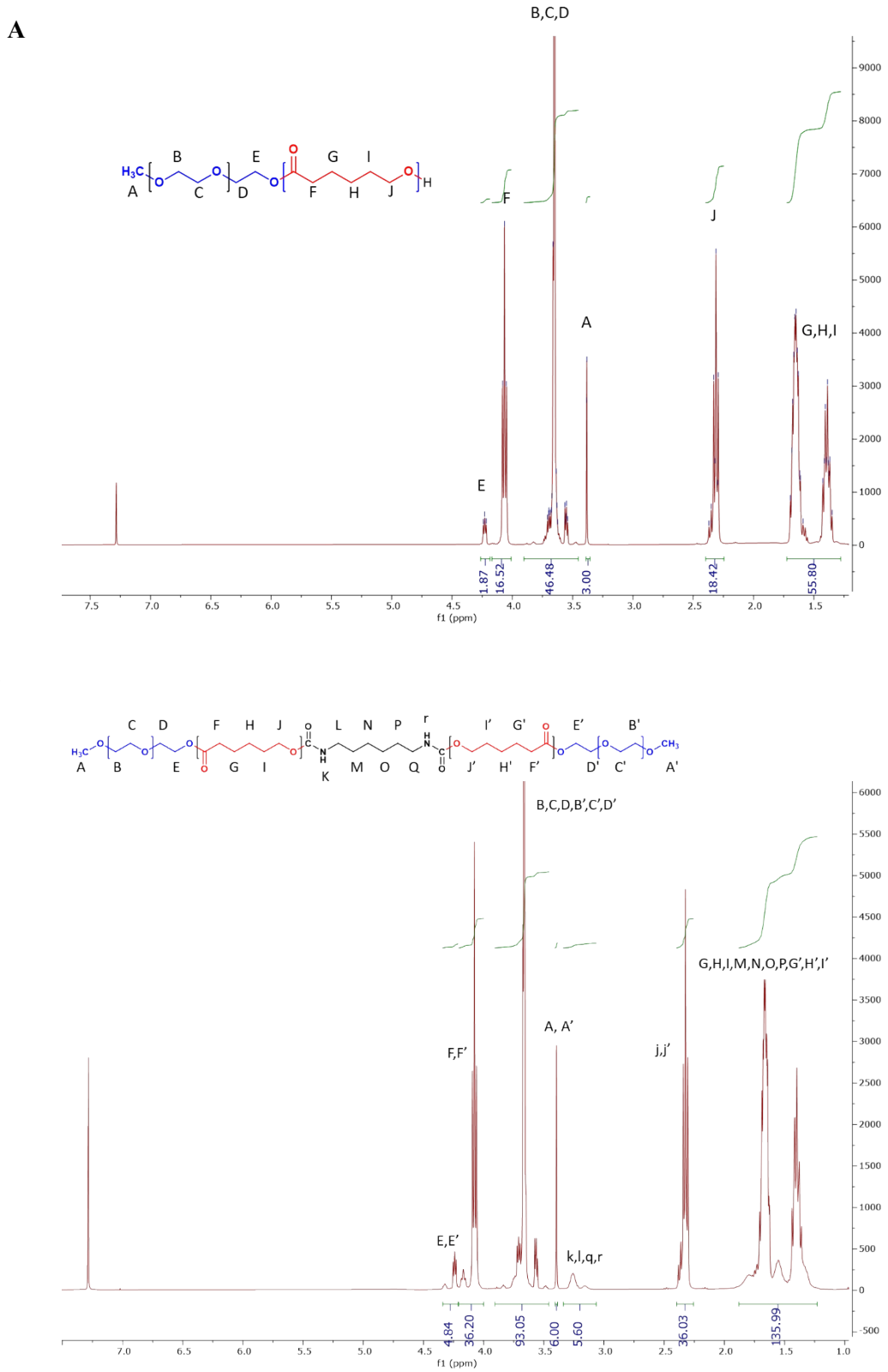

Fig. S1. ^{1}H NMR for PECE synthesis. (A) Diblock mPEG-PCL (^{1}H NMR (400 MHz, CDCl3) δ 4.28 – 4.15 (m, 2H), 4.06 (t, J = 6.7 Hz, 17H), 3.91 – 3.44 (m, 46H), 3.38 (s, 3H), 2.42 – 2.21 (m, 18H), 1.73 – 1.28 (m, 56H)) and **(B)** methoxypoly(ethylene glycol)-poly(ε-caprolactone)-methoxypoly(ethylene glycol) (mPEG-PCL-mPEG, or PECE) triblock polymer (^{1}H NMR (400 MHz, CDCl3) δ 4.24 (s, 4H), 4.08 (t, J = 6.7 Hz, 36H), 3.89 – 3.45 (m, 94H), 3.39 (s, 6H), 3.26 (s, 6H), 2.32 (t, J = 7.5 Hz, 36H), 1.88 – 1.23 (m, 136H))

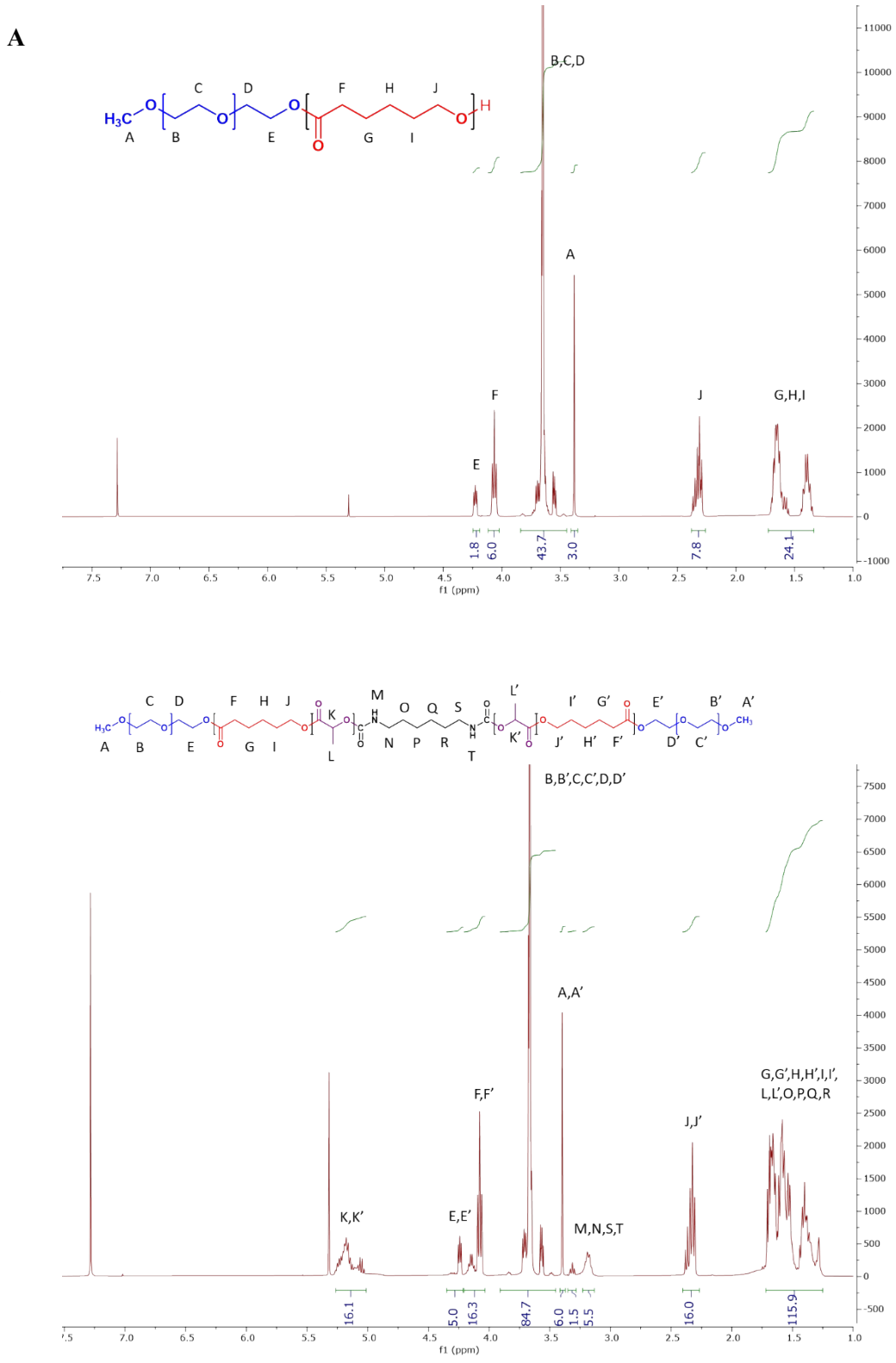

Fig. S2. ^{1}H NMR for P1 synthesis. **(A)** Diblock mPEG-PCL (^{1}H NMR (400 MHz, CDCl3) δ 4.27 – 4.19 (m, 2H), 4.07 (td, *J* = 6.7, 1.7 Hz, 6H), 3.85 – 3.42 (m, 44H), 3.38 (s, 3H), 2.40 – 2.27 (m, 8H), 1.72 – 1.32 (m, 24H)) and **(B)** P1 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.31 – 5.01 (m, 16H), 4.33 – 4.18 (m, 4H), 4.18 – 4.04 (m, 16H), 3.90 – 3.43 (m, 84H), 3.40 (s, 6H), 3.18 (d, *J* = 7.0 Hz, 6H), 2.39 – 2.25 (m, 16H), 1.74 – 1.19 (m, 116H))

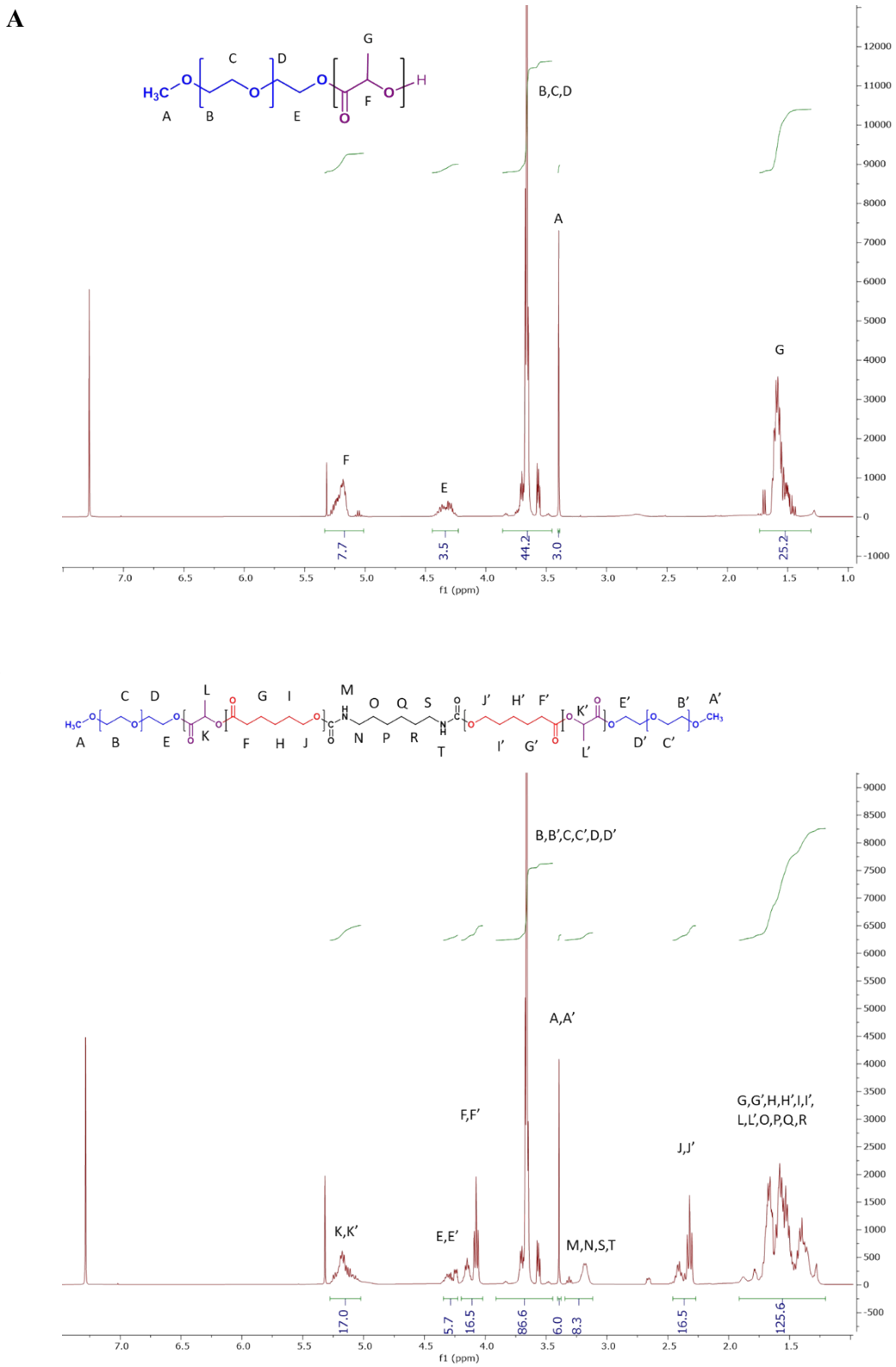

Fig. S3. ^{1}H NMR for P2 synthesis. **(A)** Diblock mPEG-PLA (^{1}H NMR (400 MHz, CDCl3) δ 5.29 – 5.09 (m, 6H), 4.44 – 4.23 (m, 3H), 3.86 – 3.46 (m, 13H), 3.40 (s, 3H), 1.73 – 1.32 (m, 24H)) and **(B)** P2 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.29 – 5.03 (m, 16H), 4.36 – 4.20 (m, 4H), 4.11 (dt, *J* = 31.1, 6.3 Hz, 16H), 3.90 – 3.44 (m, 86H), 3.39 (s, 6H), 2.48 – 2.28 (m, 16H), 1.90 – 1.16 (m, 126H)).

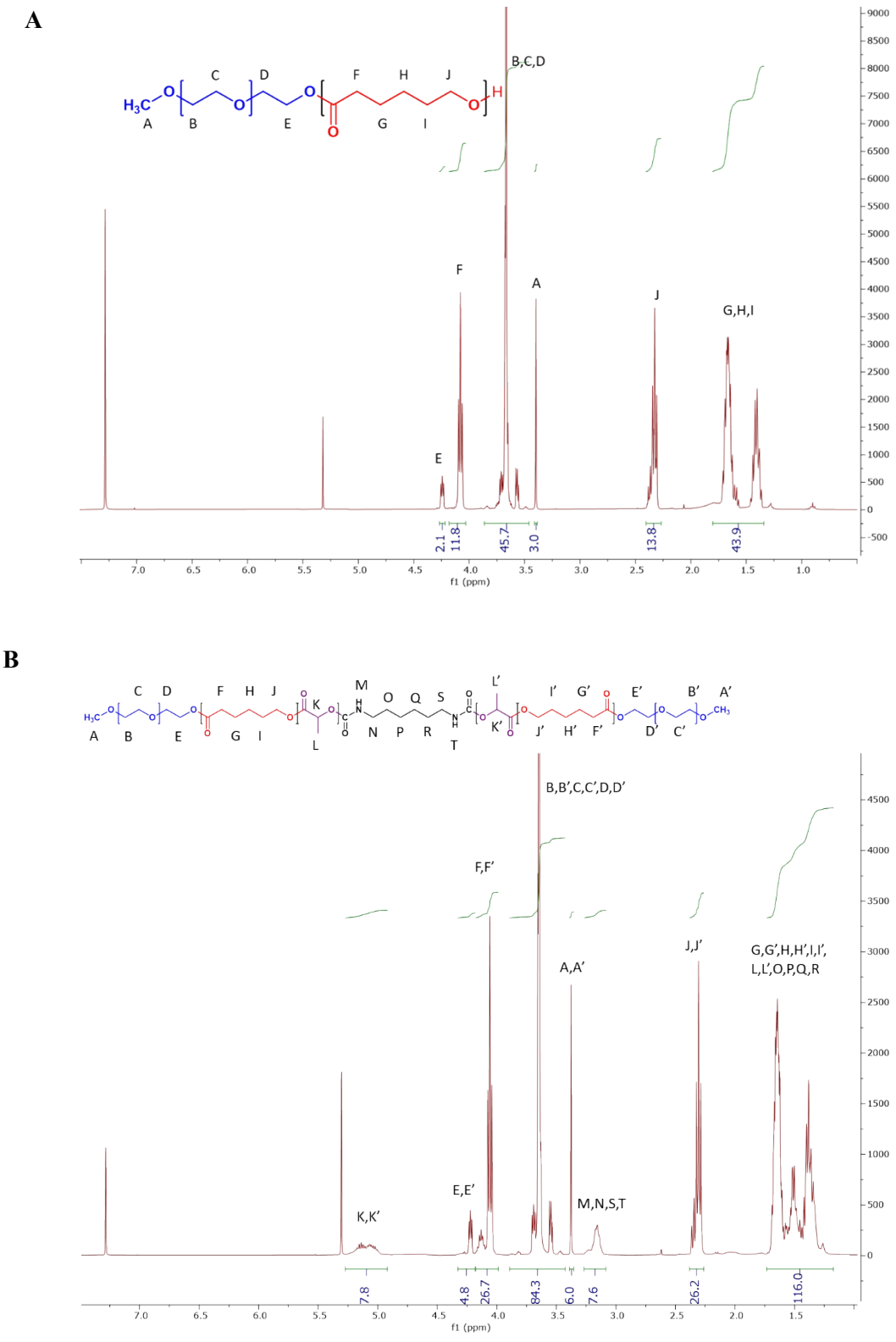

Fig. S4. ^{1}H NMR for P3 synthesis. **(A)** Diblock mPEG-PCL (^{1}H NMR (400 MHz, CDCl3) δ 4.29 – 4.21 (m, 2H), 4.08 (td, *J* = 6.7, 2.0 Hz, 12H), 3.85 – 3.50 (m, 46H), 3.40 (s, 3H), 2.41 – 2.28 (m, 14H), 1.79 – 1.31 (m, 44H)) and **(B)** P3 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.23 – 4.97 (m, 8H), 4.26 – 4.19 (m, 4H), 4.06 (t, *J* = 6.7 Hz, 26H), 3.88 – 3.43 (m, 86H), 3.38 (s, 6H), 3.26 – 3.07 (m, 6H), 2.32 (dt, *J* = 15.2, 7.5 Hz, 26H), 1.70 – 1.19 (m, 114H)).

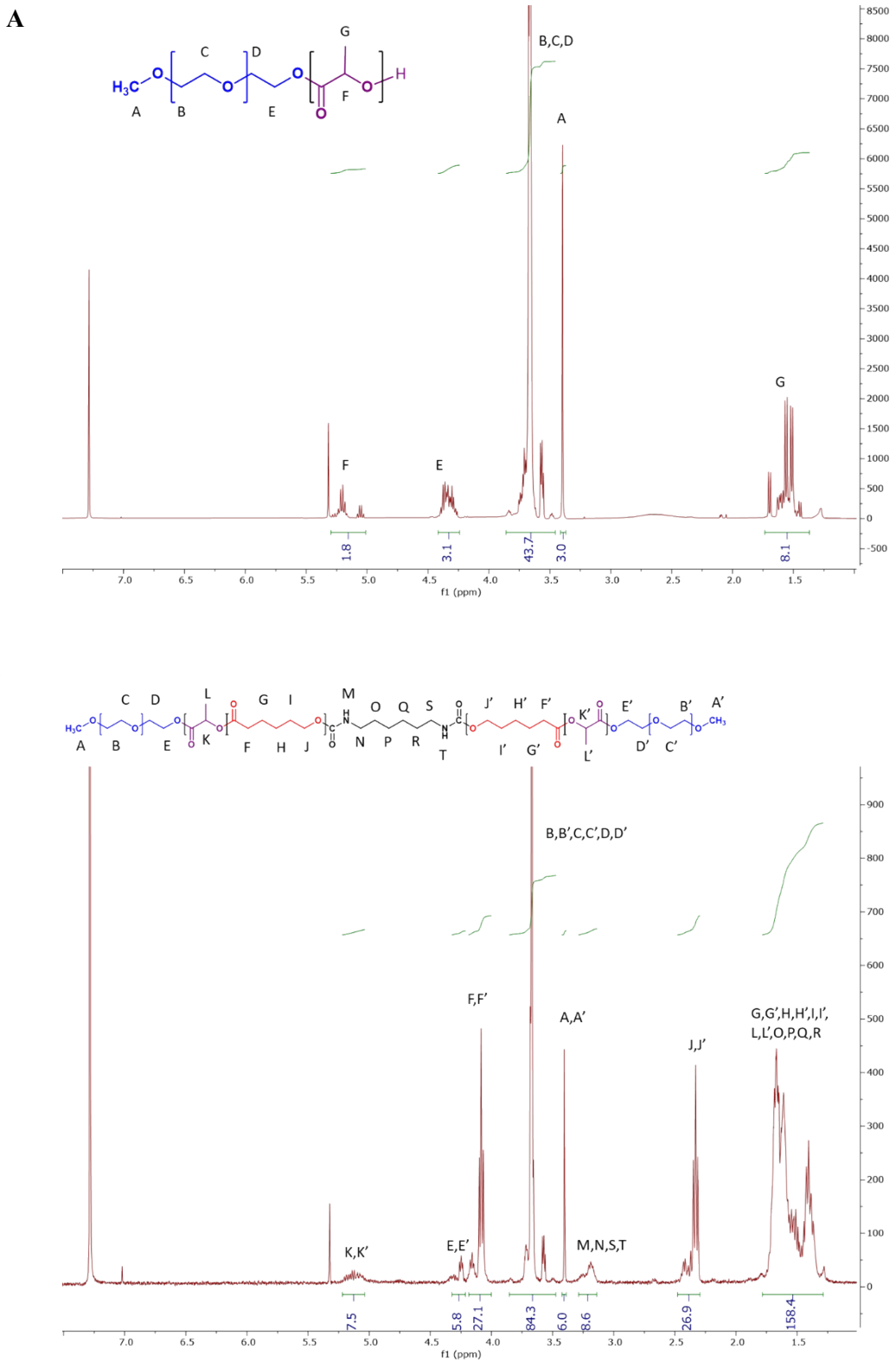

Fig. S5. ^{1}H NMR for P4 synthesis. **(A)** Diblock mPEG-PLA (^{1}H NMR (400 MHz, CDCl3) δ 5.21 (d, J = 7.0 Hz, 2H), 4.42 – 4.25 (m, 2H), 3.88 – 3.46 (m, 44H), 3.40 (d, J = 1.0 Hz, 3H), 1.71 – 1.40 (m, 8H)) and **(B)** P4 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.21 – 5.02 (m, 8H), 4.25 (s, 4H), 4.08 (t, J = 6.7 Hz, 26H), 3.40 (s, 84H), 3.19 (s, 6H), 2.34 (q, J = 8.0 Hz, 26H), 1.80 – 1.26 (m, 158H)).

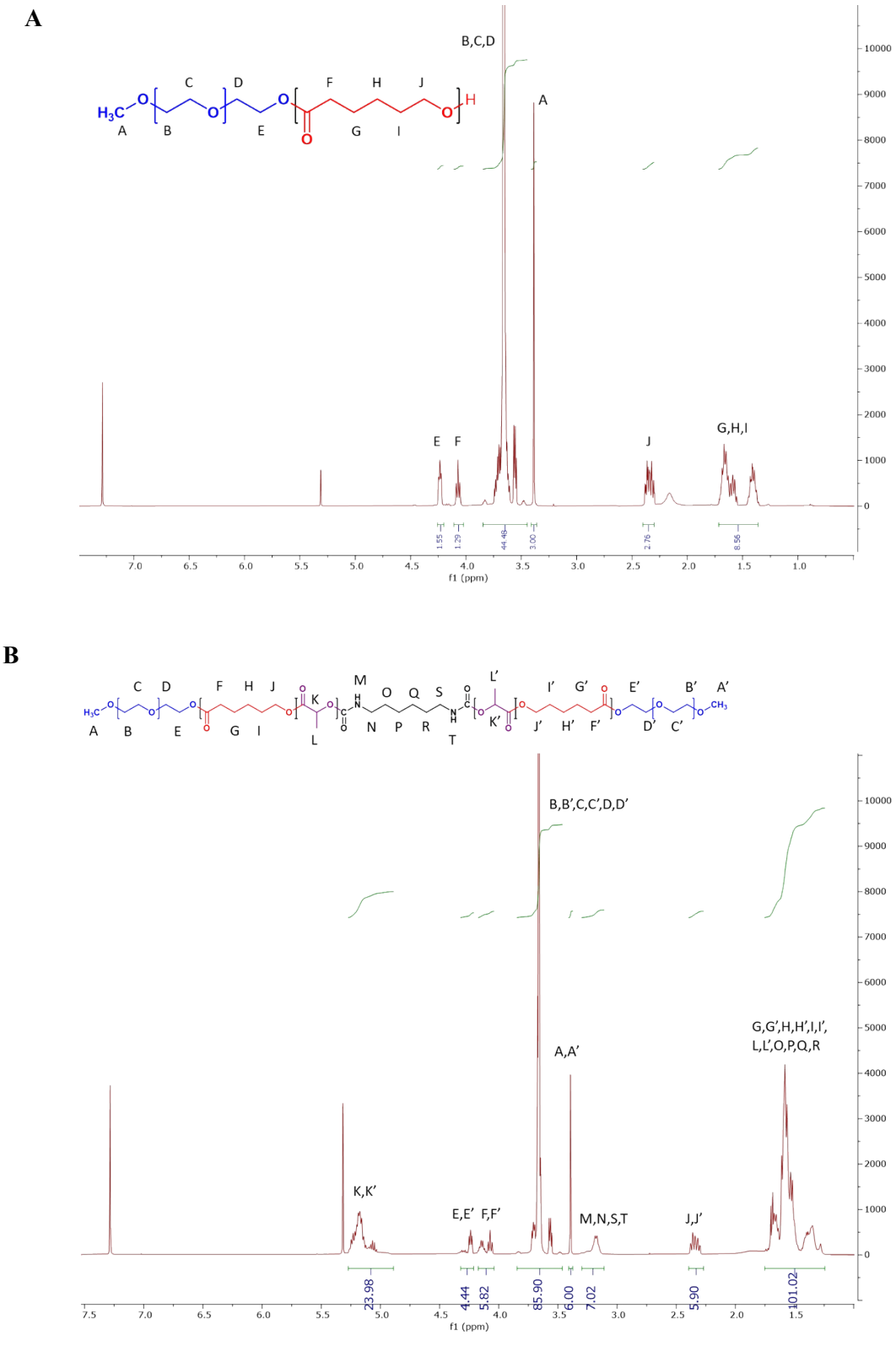

Fig. S6. ^{1}H NMR for P5 synthesis. **(A)** Diblock mPEG-PCL (^{1}H NMR (400 MHz, CDCl3) δ 4.23 (td, *J* = 4.9, 1.8 Hz, 2H), 4.07 (td, *J* = 6.7, 1.9 Hz, 2H), 3.85 – 3.43 (m, 44H), 3.39 (s, 3H), 2.40 – 2.27 (m, 2H), 1.73 – 1.32 (m, 8H)) and **(B)** P5 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.24 – 4.91 (m, 24H), 4.29 – 4.20 (m, 4H), 4.11 (dq, *J* = 25.2, 6.3 Hz, 6H), 3.85 – 3.44 (m, 86H), 3.39 (s, 6H), 3.18 (d, *J* = 6.8 Hz, 6H), 2.34 (dtd, *J* = 15.6, 7.5, 1.9 Hz, 6H), 1.75 – 1.26 (m, 102H)).

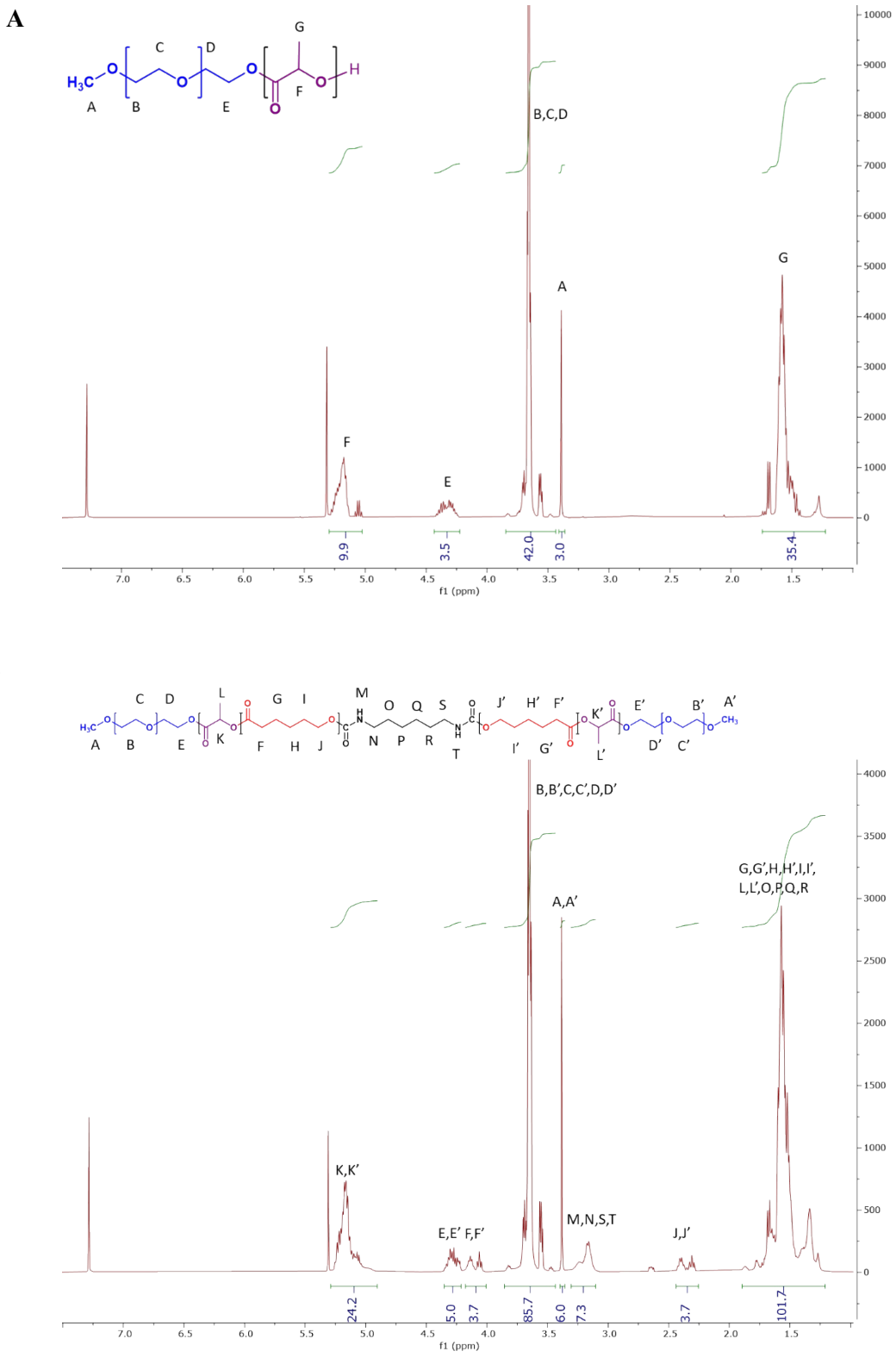

Fig. S7. ^{1}H NMR for P6 synthesis. **(A)** Diblock mPEG-PLA (^{1}H NMR (400 MHz, CDCl3) δ 5.25 – 5.02 (m, 10H), 4.35 (d, *J* = 6.7 Hz, 2H), 3.86 – 3.44 (m, 42H), 3.39 (d, *J* = 1.4 Hz, 3H), 1.59 (td, *J* = 7.3, 3.9 Hz, 36H)) and **(B)** P6 pentablock polymer (^{1}H NMR (400 MHz, CDCl3) δ 5.27 – 4.93 (m, 24H), 4.35 – 4.20 (m, 4H), 4.10 (dt, *J* = 31.2, 6.2 Hz, 4H), 3.86 – 3.44 (m, 86H), 3.38 (s, 6H), 3.17 (d, *J* = 6.9 Hz, 6H), 2.36 (dq, *J* = 33.8, 7.3 Hz, 4H), 1.89 – 1.23 (m, 102H)).

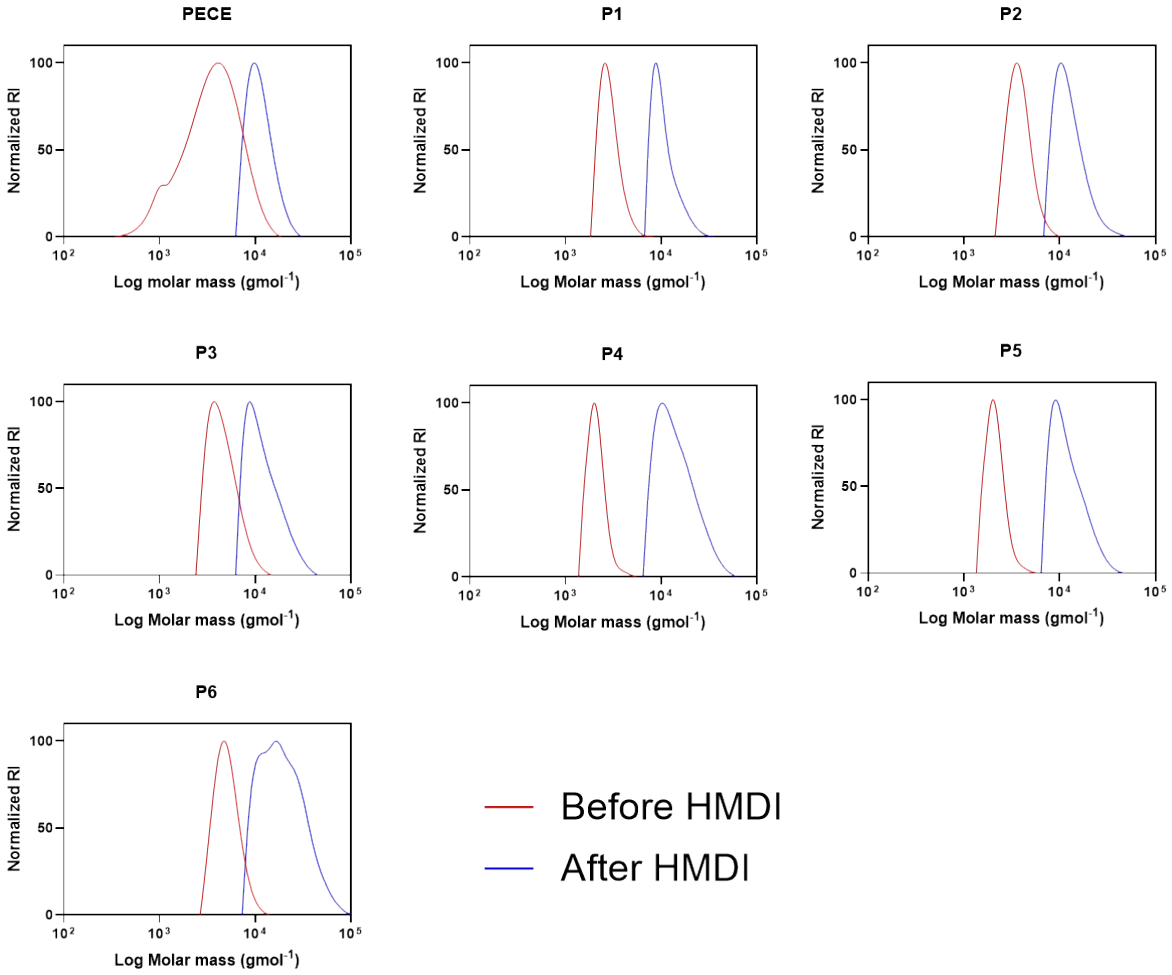

Fig. S8. GPC chromatogram before and after addition of HMDI coupling agent for all polymers. The mobile phase consisted of DMF with 0.1% (w/v) LiBr.

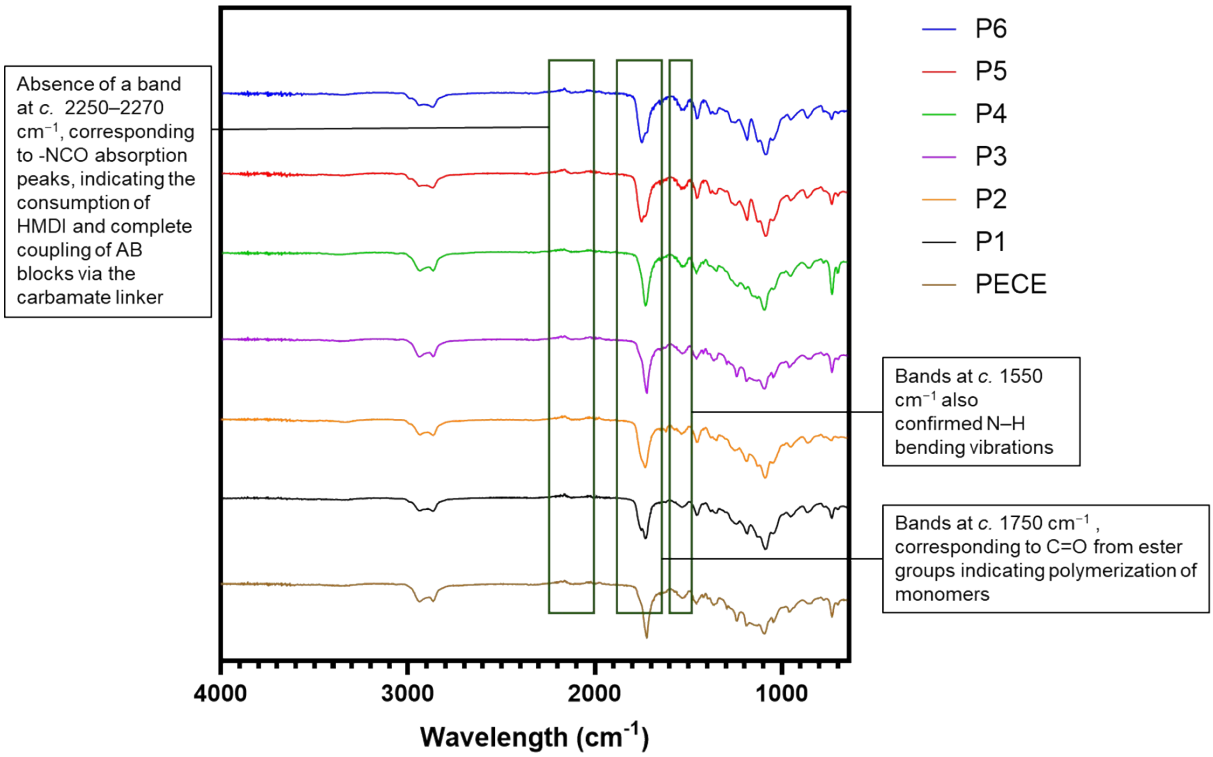

Fig. S9. FT-IR spectra for all polymers.

The drug release mechanism for the initial 60% release was evaluated by fitting the data to zero-order, first-order, Hixson-Crowell (HC), Higuchi, and Korsmeyer-Peppas models. All mathematical equations are described as follows:

The mathematical equation for zero-order kinetics employed in linear regression was:

$$C_t = C_o - kt$$

where C_o is the drug concentration at time zero, C_t is the concentration at time t, and k is the apparent release rate constant.

First-order kinetics were determined using the equation:

$$ln\, C_t = ln\, C_o - kt$$

The HC models drug release was determined using the equation:

$$\sqrt[3]{W_0} = \sqrt[3]{W_i} + kt$$

where W_0 is the initial drug amount, W_i is the remaining amount at time t, and k is the HC release rate constant.

The Higuchi model was employed with the equation:

$$Q = kt^{0.5}$$

where Q represents the fraction of drug released at time t, and k is the Higuchi release rate constant.

The Korsmeyer–Peppas release model utilised the equation:

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ is the fraction released at time t, k is the release rate constant, and n is the exponent of release which provides insights into the drug release mechanism. Values of 'n' ≤ 0.5 indicate diffusion-controlled release, while $n = 1$ signifies swelling-controlled release. Anomalous transport, involving a combination of diffusion and swelling, is suggested when $0.5 < n < 1$. Values of 'n' exceeding 1 imply that polymer erosion or degradation contributes significantly to the release process.

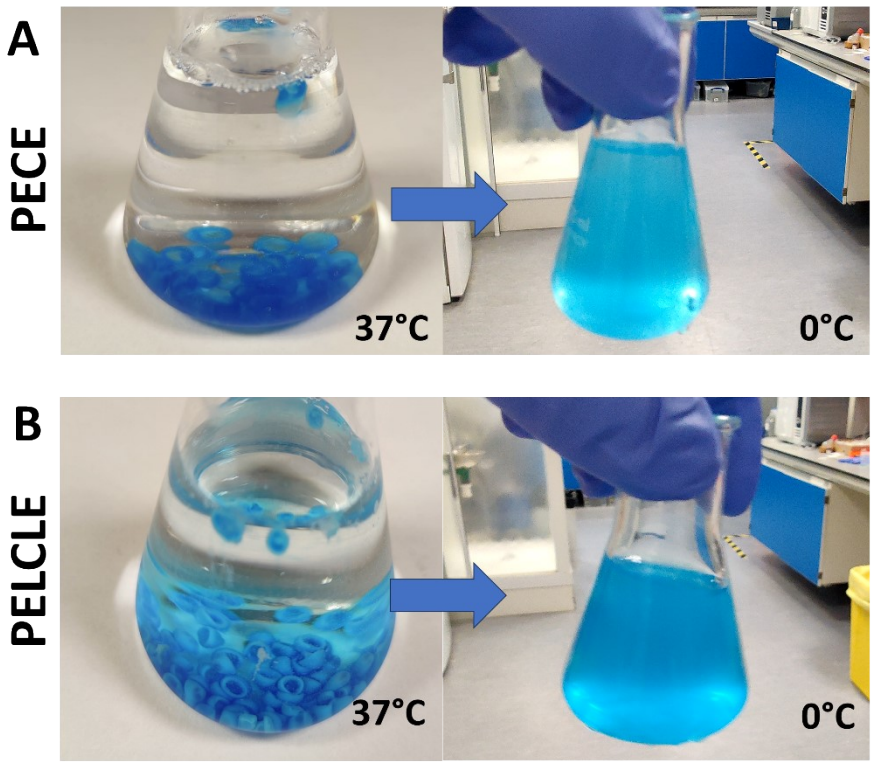

Fig. S10. Observing the controlled release of Cy5.5 from hydrogels loaded with the fluorophore, cooled down on ice (0 °C), subsequent to the injection of (A) PECE and (B) PELCLE hydrogels at 37 °C.

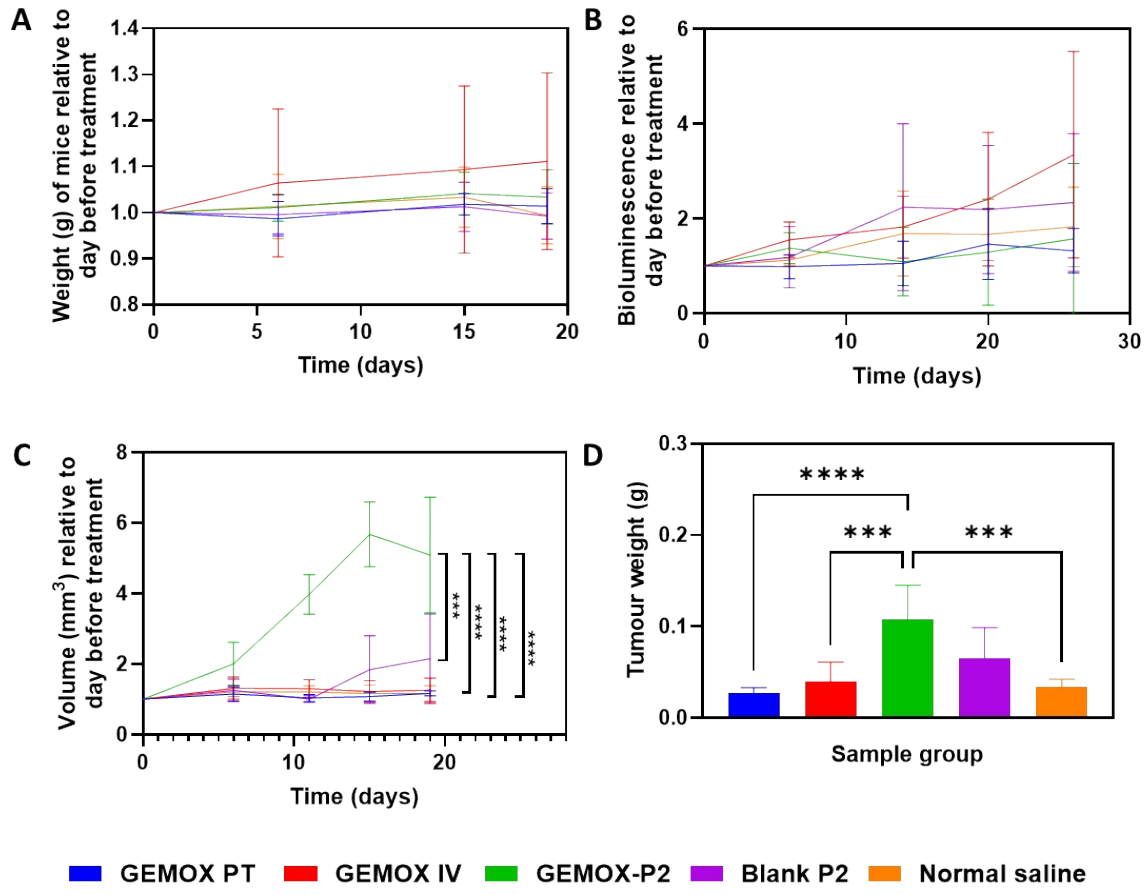

Fig. S11. Assessment of Treatment Effects on Mouse Weight, Tumour Bioluminescence, Volume, and *Ex Vivo* Tumour Weights. (A) Mouse weight relative to the day before treatment injection**. (B)** Bioluminescence of tumours relative to the day before treatment injection. **(C)** Tumour volume relative to the day before treatment injection *(n=6 ±SD)* (****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 ordinary one-way ANOVA with Tukey's post-analysis test).. **(D)** Tumour weights *ex vivo* at the termination of the study *(n=6 ±SD)* (****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 ordinary one-way ANOVA with Tukey's post-analysis test).

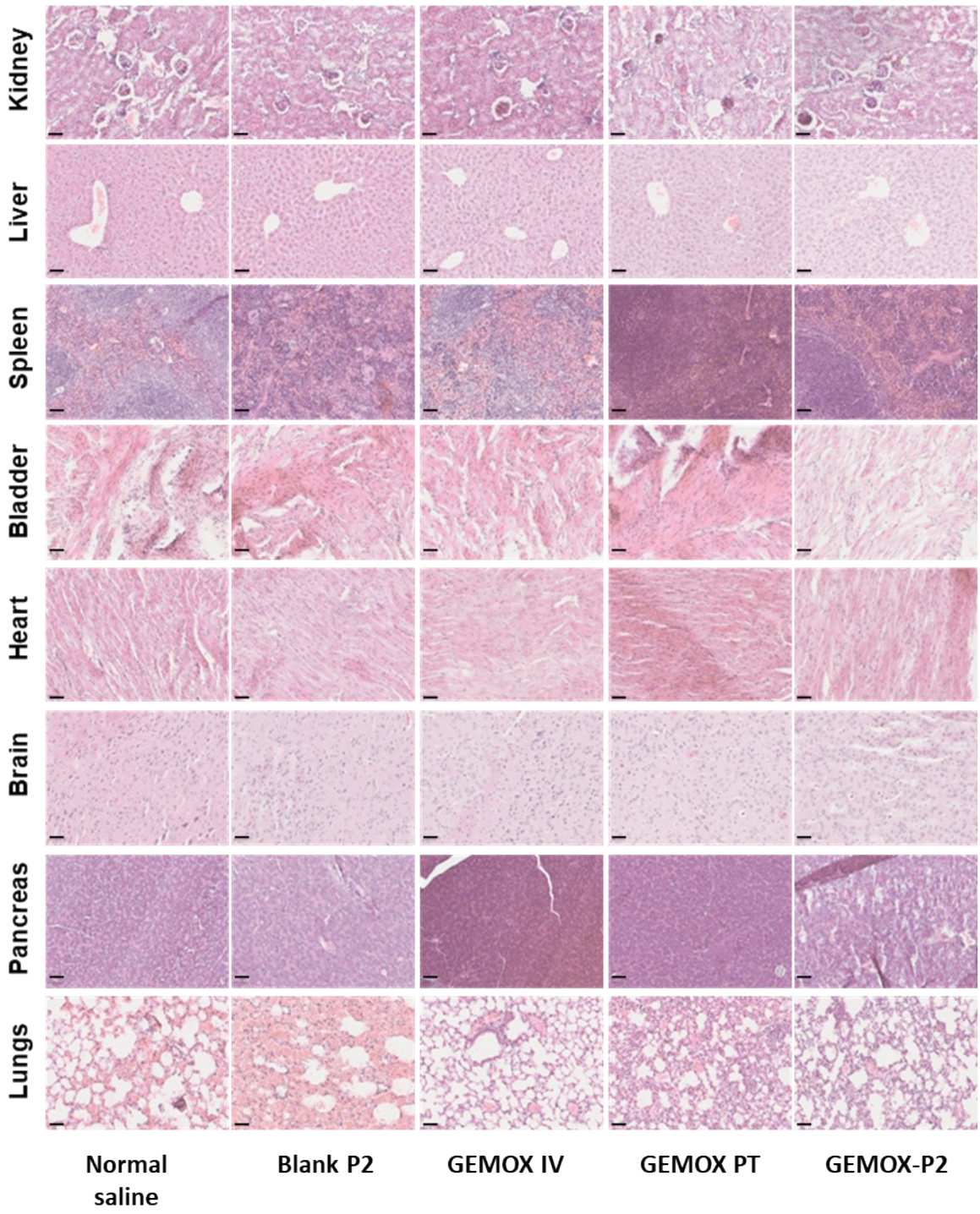

Fig. S12. Systemic toxicity assessed using H&E staining for main organs upon different treatments (Scale bar = 50 μm).

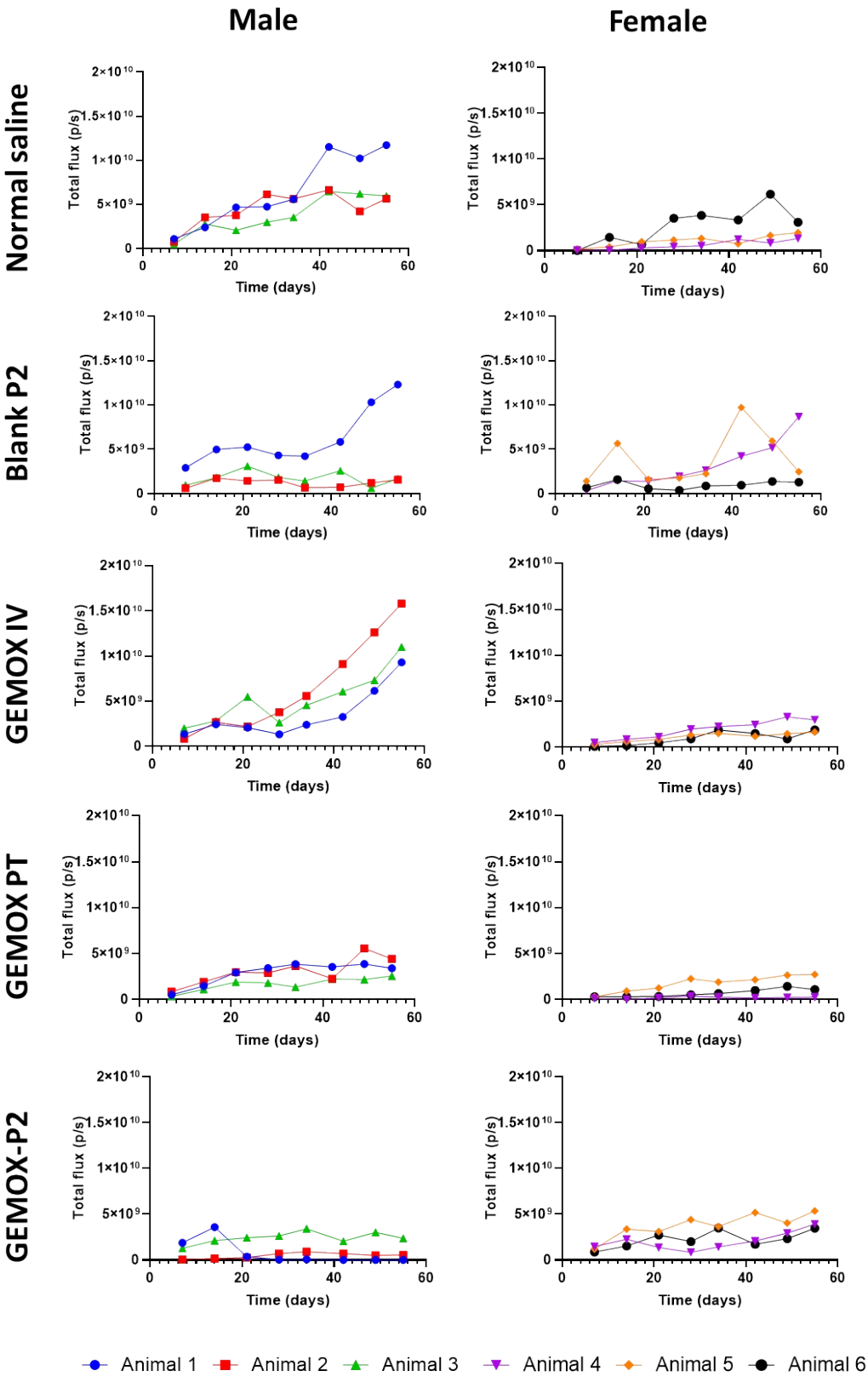

Fig. S13. Effect of treatment on tumour growth in male and female mice, as measured by bioluminescence.